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1. REPORT DATE <b>2003</b>	2. REPORT TYPE	3. DATES COVERED <b>00-00-2003 to 00-00-2003</b>		
4. TITLE AND SUBTITLE <b>Base-Dependent Competitive Adsorption of Single-Stranded DNA on Gold</b>			5a. CONTRACT NUMBER	
			5b. GRANT NUMBER	
			5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)			5d. PROJECT NUMBER	
			5e. TASK NUMBER	
			5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>Naval Research Laboratory, 4555 Overlook Avenue SW, Washington, DC, 20375</b>			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)			10. SPONSOR/MONITOR'S ACRONYM(S)	
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release; distribution unlimited</b>				
13. SUPPLEMENTARY NOTES				
14. ABSTRACT				
15. SUBJECT TERMS				
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT <b>Same as Report (SAR)</b>	18. NUMBER OF PAGES <b>2</b>
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>	19a. NAME OF RESPONSIBLE PERSON	

## Base-Dependent Competitive Adsorption of Single-Stranded DNA on Gold

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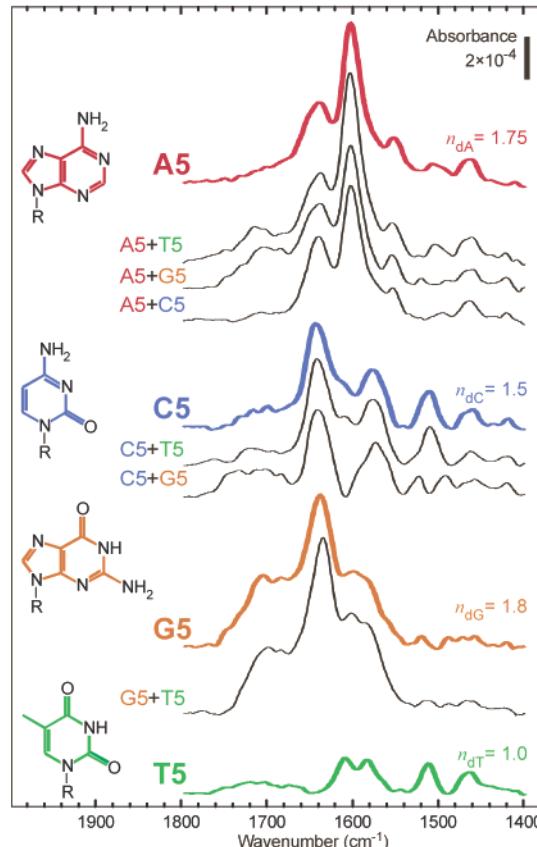
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Single-stranded DNA probes (ssDNA) immobilized on gold surfaces are a common element in many biotechnology and nanotechnology applications.<sup>1–3</sup> Although recent studies have shown that different DNA bases and homo-oligonucleotides interact differently with Au surfaces,<sup>4,5</sup> competitive interactions among the bases, which will occur in most practical applications, have not been systematically addressed. Here, we examine room-temperature adsorption of homo-oligonucleotides onto polycrystalline Au films, including competitive adsorption between all possible pairs of such unmodified oligomers. Homo-oligonucleotides serve as a model system that allows us to systematically study the base- and length-dependence of ssDNA–Au interactions, while maintaining most properties of practical ssDNA probes. We characterize the adsorption ex-situ using Fourier transform infrared (FTIR) spectroscopy and X-ray photoelectron spectroscopy (XPS), two methods that together provide unambiguous spectral signatures of the different bases and allow absolute surface densities to be determined.<sup>6</sup>

We produced ssDNA films by soaking clean Au substrates in 1  $\mu$ M solutions of unmodified homo-oligonucleotides (1 M NaCl–TE buffer) for 20 h (see Supporting Information). Adsorption of each individual homo-oligonucleotide produces a specific FTIR signature (Figure 1) and a different surface density as determined by XPS. These FTIR spectra can be compared with those following competitive adsorption from equimolar mixtures of pairs of unmodified ssDNA 5-mers [(dA)<sub>5</sub>, etc.]. For mixtures that contain (dA)<sub>5</sub>, the spectra are dominated by peaks characteristic of (dA)<sub>5</sub> at 1639, 1603, and 1553  $\text{cm}^{-1}$ . Similarly, the spectra for G + T and C + T combinations are dominated by signatures of (dG)<sub>5</sub> and (dC)<sub>5</sub>, respectively. From these results, it is clear that (dA)<sub>5</sub> has the highest adsorption affinity on Au and (dT)<sub>5</sub> has the lowest [consistent with prior conclusions<sup>5</sup> regarding oligo(dT)].

Competitive adsorption results for the two complementary combinations, C + G and A + T, require special consideration given that adsorbed duplexes have spectral characteristics different from those of homo-oligonucleotides.<sup>7</sup> As expected, the C + G spectrum is not simply a combination of (dG)<sub>5</sub> and (dC)<sub>5</sub> features, but also includes those from the duplex. On the basis of spectra for a range of adsorption times and oligomer concentrations, we find that the (dC)<sub>5</sub>•(dG)<sub>5</sub> duplex has a lower adsorption affinity than the individual oligomers and that the affinity of (dC)<sub>5</sub> is marginally higher than that of (dG)<sub>5</sub>. A lower affinity is anticipated for duplexes, where the relatively reactive bases are sequestered from the surface.

Because the adsorption of (dT)<sub>5</sub> alone creates relatively weak FTIR spectral features, it is difficult to determine its relative contribution to the A + T spectrum. To obtain a clearer picture of A + T mixtures, we performed additional experiments using 25-mers, which produce thicker (dT)<sub>25</sub> films with spectral features of comparable intensity to those of (dA)<sub>25</sub> (Figure 2). Adsorbing a

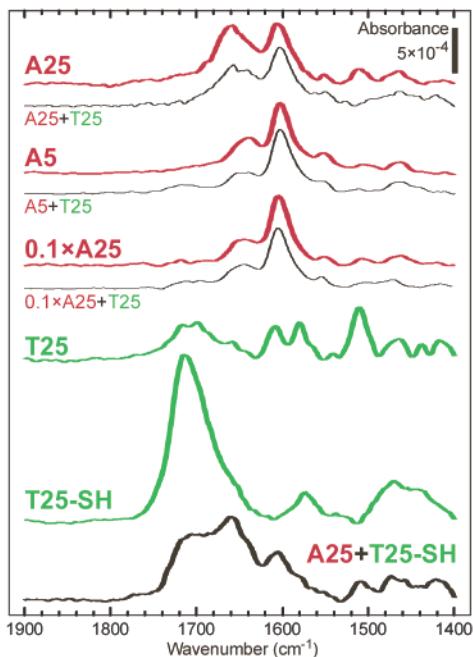


**Figure 1.** FTIR spectra of ssDNA films adsorbed on Au from aqueous solutions. Reference spectra for films of individual 5-mer homo-oligonucleotides are shown along with spectra following adsorption from their equimolar mixtures. The corresponding surface density,  $n$ , is indicated for each of the homo-oligonucleotides ( $\times 10^{14}$  nucleotides/ $\text{cm}^2$ ).

complementary mixture of unmodified A + T 25-mers onto Au results in a spectrum nearly identical to that of the (dA)<sub>25</sub> alone, with the peak at 1714  $\text{cm}^{-1}$  characteristic of the carbonyl group in thymine notably absent. This observation holds true even when an equimolar concentration of shorter (dA)<sub>5</sub> is combined with (dT)<sub>25</sub>, a case where the greater concentration of T might be expected to enhance its adsorption. Clearly, oligo(dA) has a much greater affinity for Au than does oligo(dT). Moreover, there is no evidence for adsorbed hybrids; therefore, the adsorption affinity of oligo(dA) must also be much greater than that of oligo(dA)•oligo(dT) hybrids.

Combining the results of the competitive adsorption experiments, we find the homo-oligonucleotides adsorb on Au with relative affinity A > C  $\geq$  G > T. It is important to differentiate between the relative adsorption affinities observed during competitive experiments and the relative chemisorption energies of individual DNA bases or nucleosides. As indicated by the heats of desorption

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**Figure 2.** FTIR spectra of ssDNA films on Au following competitive adsorption from mixtures of  $(dA)_n$  and  $(dT)_n$ . Spectra for  $(dA)_{25} + (dT)_{25}$ ,  $(dA)_5 + (dT)_{25}$ ,  $0.1 \times (dA)_{25} + (dT)_{25}$ , and  $(dA)_{25} + (dT)_{25}\text{-SH}$  mixtures are shown. The spectra following adsorption of  $(dA)_{25}$ ,  $(dA)_5$ ,  $0.1 \times (dA)_{25}$ ,  $(dT)_{25}$ , and  $(dT)_{25}\text{-SH}$  alone are shown for reference.

from gold,<sup>4</sup> the bicyclic purines adsorb more strongly than the monocyclic pyrimidines in the sequence G > A > C > T. The relative surface densities on gold also follow this trend, as shown by our results and others.<sup>4,5</sup> Because the affinity series determined in our work is different, that is, A > C  $\geq$  G > T, we conclude that the energetics of competitive adsorption is not governed solely by the heat of desorption.

A surprising effect of the high adsorption affinity of oligo(dA) on Au is the apparent absence of duplex adsorption from A + T mixtures. One would certainly expect oligo(dA)·oligo(dT) duplexes to be present in such a solution, but one might postulate that adsorption is dominated by residual free oligo(dA). Additional experiments reveal that this is not the case. To suppress the concentration of free  $(dA)_{25}$  by several orders of magnitude,<sup>8</sup> we hybridized a solution containing  $1.0 \mu\text{M}$   $(dT)_{25}$  and  $0.1 \mu\text{M}$   $(dA)_{25}$  (see Supporting Information). As shown in Figure 2, the resulting film is nearly identical to that observed from the same dilution of  $(dA)_{25}$ , with negligible contributions from adsorbed  $(dT)_{25}$  and no evidence of adsorbed (dA)·(dT) hybrids. We conclude that the interaction of oligo(dA) with Au is so strong that it denatures (dA)·(dT) duplexes present in solution.

To examine the relevance of our results to applications that use thiol-modified ssDNA on Au,<sup>1</sup> unmodified  $(dA)_{25}$  was mixed with thiolated  $(dT)_{25}$ . Thiolated  $(dT)_{25}$  probes adsorb on Au as randomly coiled molecules each anchored via the thiol group.<sup>6</sup> Thus, one would expect this mixture to result in  $(dA)_{25}\text{-}(dT)_{25}$  duplexes adsorbed via the thiolated 5' end of the  $(dT)_{25}$ . However, the extraordinary affinity of oligo(dA) for Au again produces a film composed largely of adsorbed  $(dA)_{25}$  (Figure 2, bottom).

Several factors must be responsible for the observed variation in the adsorption affinities, because no single structural or chemical characteristic of ssDNA probes can explain our results. The adsorption affinity is essentially independent of the oligonucleotide length. Because the backbone and sugar are common to all oligonucleotides, the variation in affinity must be specific to the structure of DNA bases. Given the different behavior of the purines, A and G, it is

unlikely that the relative adsorption affinities are determined by the specific interaction of one functional group (e.g., an exocyclic amine) with the surface. The complexity of the FTIR and XPS spectra as a function of oligomer surface density (the subject of a forthcoming publication) indeed indicates that most of the nucleotides chemisorb on Au through multiple binding sites. In addition, differences in the formation of secondary structure in the homo-oligonucleotides (e.g., in  $(dG)_5$ )<sup>9</sup> may affect the observed affinities.

For the exceptional case of  $(dA)_n$ , both FTIR and XPS indicate that during the initial adsorption almost every base within each molecule chemisorbs on the surface, even for 25-mers. Two binding geometries have been previously suggested for adenine on metal surfaces: the N6 exocyclic amino group<sup>10</sup> and the N7 atom.<sup>11</sup> Although the precise binding geometry for dA nucleotides on Au is unknown, we speculate that the exceptionally high adsorption affinity of oligo(dA) is due to adenine adsorption via both of the above N groups in coordination.

In summary, from competitive adsorption experiments on Au surfaces, we find the relative adsorption affinity of DNA bases to be A > C  $\geq$  G > T, with the adsorption of oligo(dA) strongly dominating over the other oligonucleotides. Its high adsorption affinity allows oligo(dA) to compete effectively against chemisorption of thiolated oligo(dT) and causes hybridized oligo(dA)·oligo(dT) duplexes to denature in the presence of Au. This remarkable affinity has numerous practical implications. Clearly, probe and target oligonucleotides may be affected in systems where Au substrates, electrodes, or nanoparticle labels are used. For example, the effective competition of oligo(dA) against a thiolated oligo(dT) explains some puzzling results of a recent study where ferrocene-labeled, thiolated  $(dT)_{20}$  was hybridized with unmodified  $(dA)_{20}$  and deposited on Au, but did not produce the expected electrochemical signal.<sup>12</sup> Our results suggest that the unlabeled  $(dA)_{20}$  preferentially adsorbed undetected onto the electrode. Our results also imply that the adsorption of messenger RNAs with polyA tails should be particularly favorable on Au surfaces. Finally, we suspect similar competitive adsorption effects occur on other substrates, possibly with different base dependences.

**Acknowledgment.** H.K.-S. thanks Drs. P. M. Vallone and T. H. Huang (NIST) for MALDI of DNA samples and advice on DNA hybridization, respectively. D.Y.P. and L.J.W. are grateful for stimulating discussions with Drs. L. A. Baker, T. D. Clark, S. E. Kooi, J. N. Russell, Jr., and P. E. Sheehan (NRL). Work at NRL was supported by ONR and AFOSR.

**Supporting Information Available:** Materials and methods (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JA035756N